REFERENCE
Pouliquen,H.; Fauconnet,V.; Morvan,M.-L.; Pinault,L. Determination of warfarin in the yolk and the white of hens' eggs by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 143–148.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 250 μL 1 M HCl to 500 μL microsomal incubation, extract with 3 mL MTBE, evaporate the organic layer under nitrogen, reconstitute the residue in 100 μL MeCN: water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 Novapak C18
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM acetic acid adjusted to pH 4.8 with ammonium hydroxide.)
Flow rate: 1
Injection volume: 120
Detector: Radioactivity, Inus β-Ram using Inus Tru-Count scintillation fluid at a flow rate of 5 mL/min

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

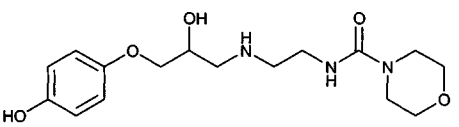
REFERENCE
Obach,R.S. Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol, *Drug Metab.Dispos.*, **1997**, *25*, 1359–1369.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm), dilute the filtrate with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:10 mM pH 4.7 acetate buffer 50:50
Detector: UV 214

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

Xamoterol



Molecular formula: $C_{16}H_{25}N_3O_5$
Molecular weight: 339.39
CAS Registry No.: 81801-12-9, 73210-73-8 (hemifumarate)
Merck Index: 10189

SAMPLE
Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with MeOH and water. 1 mL Plasma + 2 mL pH 7.0 phosphate-citrate buffer, add to the SPE cartridge, wash with 10 mL water, wash with 5 mL MeOH:water 10:90, elute with 2 mL MeOH. Evaporate the eluate to dryness at 60°, reconstitute with 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 5 μm Hypersil-ODS
Mobile phase: MeOH:THF:30 mM perchloric acid 8:0.6:91.4
Flow rate: 1.5
Injection volume: 100
Detector: E, Bioanalytical Systems Model LC-4A, TL-5A thin-layer glassy carbon electrode + 0.85 V, RE-1 Ag/AgCl reference electrode

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CHROMATOGRAM
Retention time: 8
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, acetazolamide, caffeine, chlordiazepoxide, cimetidine, codeine, diazepam, digoxin, docusate sodium, flurazepam, furosemide, heparin, hydrochlorothiazide, meclofenamate, methyldopa, nitroglycerin, quinidine, simethicone, spironolactone, thioridazine, triamterene, warfarin
Interfering: aspirin, benzyl alcohol, hydralazine

KEY WORDS
plasma; SPE

REFERENCE
Davis,P.C. Determination of xamoterol in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1987**, *417*, 233–235.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or 100 μL urine + 40 ng prenalterol + 5 mL 0.1% disodium EDTA + 100 μL 1 M pH 6.5 ammonium acetate, add to the ion-exchange column, wash with two 10 mL portions of water, elute with 3 mL 1 M ammonium hydroxide. Evaporate the eluate to dryness under reduced pressure at 50°, reconstitute with 250 μL ice-cold 100 mM perchloric acid, centrifuge at 4° at 10000 g for 10 min, inject a 100 μL aliquot of the supernatant. (Prepare an ion-exchange column by packing 50-100 mesh Bio-Rex 70 Na+ cation-exchange resin (Bio-Rad) into a 40 × 10 column, wash with 3 M HCl, 3 M NaOH, 3 M acetic acid, 1 M pH 6.5 ammonium acetate, and water.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:10 mM perchloric acid 15:85
Flow rate: 2
Injection volume: 100
Detector: F ex 190 em 320-400 (filter)

CHROMATOGRAM
Retention time: 6.9
Internal standard: prenalterol (5.8)
Limit of detection: 10 ng/mL (urine), 1 ng/mL (plasma)

OTHER SUBSTANCES
Simultaneous: albuterol, fenoterol
Noninterfering: atropine, clonidine, diazepam, nitrazepam, prazosin, quinidine

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KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Oddie,C.J.; Jackman,G.P.; Bobik,A. Measurement of xamoterol in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *308*, 370–375.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

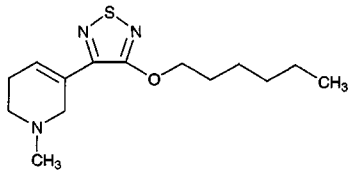
CHROMATOGRAM
Retention time: k' 1.13, k' 2.27 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

Xanomeline



Molecular formula: $C_{14}H_{23}N_3OS$
Molecular weight: 281.42
CAS Registry No.: 131986-45-3, 141064-23-5 (oxalate), 152854-19-8 (tartrate)
Merck Index: 10190

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 500 mg C18 Bond-Elut SPE cartridge with MeOH and water. Mix 2 mL microsomal incubation with 4.5 mL ice-cold MeOH, centrifuge at 4000 g for 10 min, dilute the supernatant with 15 mL water. Add a 2 mL aliquot to the SPE cartridge, wash with

2 mL water, elute with 2 mL MeOH:25% aqueous ammonia 96:4, evaporate the eluate to dryness under reduced pressure, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm ChromSpher Si (Chrompack)
Mobile phase: n-Heptane:2-propanol:water:25% ammonia 90:10:0.075:0.075 (A) or n-heptane:2-propanol:MeOH:25% ammonia 50:50:10:1 (B)
Detector: UV 295; MS, VG TRIO 1000, particle beam interface at 50°, helium at 25-30 psi, ion source 200°, positive ionization mode, electron current 150 μA, electron energy 70 eV

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
normal phase; rat; liver; SPE

REFERENCE
Andersen,J.V.; Hansen,K.T. Normal-phase liquid chromatography-particle-beam mass spectrometry in drug metabolism studies of the dopamine receptor antagonist Odapipam and the muscarine M1 receptor agonist Xanomeline, *Xenobiotica*, **1997**, *27*, 901–912.

SAMPLE
Matrix: perfusate
Sample preparation: Inject an aliquot of perfusate directly onto the column.

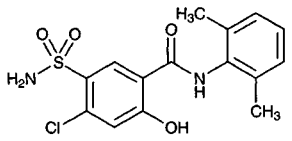
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeCN:buffer 40:60 (Buffer was 30 mM tetramethylammonium hydroxide adjusted to pH 3.0 with perchloric acid.)
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: UV 295

CHROMATOGRAM
Limit of detection: 100 nM

KEY WORDS
liver

REFERENCE
Andersen,J.V.; Hansen,K.T. Normal-phase liquid chromatography-particle-beam mass spectrometry in drug metabolism studies of the dopamine receptor antagonist Odapipam and the muscarine M1 receptor agonist Xanomeline, *Xenobiotica*, **1997**, *27*, 901–912.

Xipamide



Molecular formula: $C_{15}H_{15}ClN_2O_4S$
Molecular weight: 354.81
CAS Registry No.: 14293-44-8
Merck Index: 10212
Lednicer No.: 2 93

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL dichloromethane:2-propanol 75:25, shake for 10 min. Centrifuge at 2000 g for 10 min at 4°. Remove the organic phase and evaporate it to

dryness under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL mobile phase, mix for 10 s. Centrifuge at 6500 g for 10 min. Inject a 40 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm Supelcosil LC-18 (Supelco)
Mobile phase: n-Propanol:buffer 5:95 (Buffer was 50 mM sodium dodecyl sulfate in 10 mM pH 5.8 sodium phosphate buffer.)
Flow rate: 1.3
Injection volume: 40
Detector: UV 225

CHROMATOGRAM
Retention time: 8.58
Internal standard: xipamide

OTHER SUBSTANCES
Extracted: albuterol, atenolol, chlorthalidone

KEY WORDS
plasma; xipamide is IS

REFERENCE
Giachetti,C.; Tenconi,A.; Canali,S.; Zanolo,G. Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography. Application to pharmacokinetic studies in man, *J.Chromatogr.B*, **1997**, *698*, 187–194.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

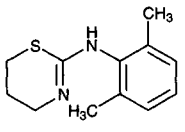
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 218.1

CHROMATOGRAM
Retention time: 18.823

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Xylazine



Molecular formula: $C_{12}H_{16}N_2S$
Molecular weight: 220.34
CAS Registry No.: 7361-61-7, 23076-35-9 (HCl)
Merck Index: 10213
Lednicer No.: 2 307

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 10 µL 100 µg/mL doxapram in MeOH + 1 mL 50 mM pH 11 borax buffer, vortex for 5 s, add 10 mL chloroform, shake for 10 min, centrifuge at 11400 g for 10 min, filter (Whatman No. 1 PS phase-separating paper). Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 100 µL mobile phase, inject the whole sample.

HPLC VARIABLES

Column: 300 × 4 10 µm µBondapak C18
Mobile phase: MeOH:water:heptanesulfonic acid (Pic B7) 45:55:0.2 containing 2% glacial acetic acid
Flow rate: 2
Injection volume: 100
Detector: UV 225

CHROMATOGRAM

Retention time: 4
Internal standard: doxapram (5.5)
Limit of detection: 20 ng/mL

KEY WORDS

plasma; sheep

REFERENCE

Alvinerie,M.; Toutain,P.L. Determination of xylazine in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *222*, 308–310.

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. Add 200 µL plasma to the SPE cartridge, wash with 100 mM ammonium acetate, elute with MeOH:100 mM ammonium acetate 3:1. Evaporate the eluate to dryness under reduced pressure, dissolve the residue in 200 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Hitachi gel 3056 octadecylsilica
Mobile phase: MeOH:100 mM ammonium acetate 60:40
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M1000, APCI, nebulizer 260°, vaporizer 399

CHROMATOGRAM

Retention time: 7.0
Limit of detection: 0.5-2.5 ng/mL

OTHER SUBSTANCES

Simultaneous: atipamezole, atropine, butorphanol, flumazenil, ketamine, medetomidine, midazolam

KEY WORDS
plasma; SPE; dog

REFERENCE
Kanazawa,H.; Nagata,Y.; Matsushima,Y.; Takai,N.; Uchiyama,H.; Nishimura,R.; Takeuchi,A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, *631*, 215–220.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.53

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 μL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 μL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES
Guard column: 10 × 2.1 37-50 μm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 4 ng/g

OTHER SUBSTANCES
Extracted: azaperol, carazolol, acepromazine, azaperone, haloperidol, propiomazine, chlorpromazine

KEY WORDS
SPE; pig; kidney

REFERENCE
Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Cut pig kidney or liver into small pieces and homogenize. 5 g Homogenate + 10 mL MeCN, shake, vortex for 30 s, sonicate for 3 min, vortex for 30 s, sonicate for 3 min, centrifuge at 10000 g for 20 min. Add 7.5 mL supernatant + 40 mL 10% NaCl to the SPE cartridge at about 1 mL/min, do not allow cartridge to dry out, wash with 850 μL 10 mM sulfuric acid, dry with air, elute with 3.5 mL acidic MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL 10 mM sulfuric acid, vortex briefly, add 1 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, inject an aliquot of the aqueous layer. (Acidic MeCN was 1 mL 50 mM sulfuric acid in 100 mL MeCN.)

HPLC VARIABLES
Guard column: Hypersil 5 μm SAS C1
Column: 250 mm long 5 μm Hypersil SAS C1
Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate
Flow rate: 2
Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

CHROMATOGRAM
Retention time: 10
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: azaperol, acepromazine, carazolol, azaperone, haloperidol, propriomazine, chlorpromazine

KEY WORDS
SPE; pig; kidney; liver

REFERENCE
Rose,M.D.; Shearer,G. Determination of tranquilisers and carazolol residues in animal tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *624*, 471–477.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Tissuemizer) 20 g kidney and 70 mL chloroform at medium-high speed for 1-1.5 min, rinse blade with 5 mL chloroform, centrifuge at 1800 rpm for 10 min, filter (glass-fiber) supernatant, repeat extraction, rinse filter with 30 mL chloroform. Combine filtrates, add 1 mL 1 M HCl, evaporate to dryness under reduced pressure at 55°, add 25 mL MeOH, evaporate to dryness, reconstitute with 25 mL petroleum ether, add to Celite column, rinse flask with three 10 mL portions of petroleum ether, add rinses to column, rinse flask with two 50 mL portions of petroleum ether:MeOH 98.5:1.5, add to column, discard all effluent, elute with two 50 mL portions of MeOH. Evaporate eluate to dryness under reduced pressure at 55°, reconstitute with 25 mL MeOH, evaporate to dryness, reconstitute with 4-10 mL mobile phase, filter (0.45 μm), inject a 50 μL aliquot. (Petroleum ether was water saturated. Prepare Celite column as follows. Slurry 250 g Celite 545 and 800 mL HCl:water 50:50, heat on a steam bath with occasional stirring for several hours, allow to settle, pour off liquid, add 800 mL HCl: water 50:50, slurry, heat on a steam bath for several hours, allow to settle, decant liquid, wash with water until pH is neutral, wash with 250 mL MeOH, wash with 250 mL n-hexane, wash with 250 mL petroleum ether, heat on a steam bath to remove residual solvent, dry at 105°. Blend 2 g acid-washed Celite and 0.5 mL water, add to a 300 × 22 column, blend 3 g Celite and 1 mL 1 M HCl, add to the column.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:water:2 M sodium acetate:1 M acetic acid 32:64:2:2
Flow rate: 1
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
cow; pig; kidney; SPE

REFERENCE
Holland,D.C.; Munns,R.K.; Roybal,J.E.; Hurlbut,J.A.; Long,A.R. Simultaneous determination of xylazine and its major metabolite, 2,6-dimethylaniline, in bovine and swine kidney by liquid chromatography, *J.AOAC Int.*, **1993**, *76*, 720–724.

SAMPLE
Matrix: urine
Sample preparation: Condition a 1 mL 100 mg Bond-Elut cyanopropyl SPE cartridge with two 1 mL portions of MeOH and 1 mL water, do not allow to dry. 1 mL Urine + 500 μL water + 100 μL 15 μg/mL diazepam in MeOH, add to the SPE cartridge, dry under vacuum for 3 min, elute with two 250 μL portions of MeCN:methanolic HCl 50:50, inject an aliquot of the eluate. (Prepare methanolic HCl by adding 3 mL concentrated HCl to 50 mL MeOH.)

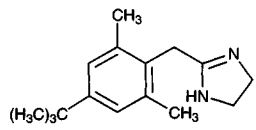
HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeCN:MeOH:buffer 30:2:50 (Buffer was 4 g/L tetramethylammonium hydroxide.)
Flow rate: 2
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 6.5
Internal standard: diazepam (9.4)
Limit of detection: 10 ng/mL

KEY WORDS
dog; SPE; pharmacokinetics

REFERENCE
Moore,C.M.; Oliver,J.S. Rapid extraction and determination of xylazine in greyhound urine using high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *491*, 519–524.

Xylometazoline



Molecular formula: $C_{16}H_{24}N_2$
Molecular weight: 244.38
CAS Registry No.: 526-36-3, 1218-35-5 (HCl)
Merck Index: 10219
Lednicer No.: 1 242

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 6.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: benzalkonium chloride, domiphen bromide, thimerosal

KEY WORDS
nasal drops

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: formulations
Sample preparation: Dilute nasal solution 10-fold with water, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. MeCN:1 mM NaOH from 10:90 to 80:20 over 25 min.
Column temperature: 25
Flow rate: 1.2
Injection volume: 10
Detector: UV 224

CHROMATOGRAM
Retention time: 14.5
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: ephedrine, naphazoline, oxymetazoline

KEY WORDS
nasal solutions

REFERENCE
De Orsi,D.; Gagliardi,L.; Cavazzutti,G.; Mediati,M.G.; Tonelli,D. Simultaneous determination of ephedrine and 2-imidazolines in pharmaceutical formulations by reversed-phase HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 3233–3242.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 5.9
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: atropine, codeine, dansylamide, dansylcadaverine, doxorubicin, methylatropine, naphazoline, noscapine

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 RSIL C18 (RSL, Eke, Belgium)
Mobile phase: MeOH:water 40:60 containing 20 mM sodium 1-octanesulfonate and 10 mM N,N-dimethyloctylamine, pH adjusted to 3.0 with orthophosphoric acid
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 40

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline, coumazoline, lidocaine, naphazoline, oxymetazoline, prednisolone, sulfadimidine, sulfanilamide, sulfathiazole, tenaphtoxaline, tetrahydrozoline, tolazoline, tramazoline

REFERENCE
De Schutter,J.A.; Van den Bossche,W.; De Moerloose,P. Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography, *J.Chromatogr.*, **1987**, *391*, 303–308.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 12.68 (A), 7.16 (B)

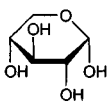
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene,

desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Xylose



Molecular formula: $C_5H_{10}O_5$
Molecular weight: 150.13
CAS Registry No.: 58-86-6
Merck Index: 10220

SAMPLE
Matrix: beverages, juice, milk
Sample preparation: Orange juice. Dilute orange juice 100-fold with water, filter (Millipore HV, 0.45 μm), dilute filtrate 10-fold, inject an aliquot. Beverages. Dilute soft drinks 1000-fold with water, inject an aliquot. Milk. Dilute 5 mL milk to 100 mL with mobile phase, filter (Millipore HV, 0.45 μm), dilute filtrate 50-fold, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Cation H (Bio-Rad)
Column: 300 × 3.8 9 μm HPX 87-H Aminex (Bio-Rad)
Mobile phase: 10 mM Sulfuric acid
Column temperature: 50
Flow rate: 0.5
Injection volume: 40
Detector: E following post-column reaction, Hewlett-Packard 1049A programmable electrochemical detector, Metrohm detector cell, cuprous oxide working electrode +550 mV, glassy carbon auxiliary electrode, Ag/AgCl (3 M KCl) reference electrode. The column effluent mixed with 200 mM NaOH pumped at 0.4 mL/min, the mixture flowed through a 220 × 0.8 single-bead string reactor packed with 0.6 mm glass beads to the detector. (Prepare cuprous oxide electrode as follows. Stir 300 mg conductive carbon cement (Gerhard Neubauer, Munster), 60 mg cuprous oxide (Fluka), and 300 μL acetone until a thick paste forms as the acetone evaporates. Pack

conductive carbon cement into the base of a 3 mm diameter cavity carbon paste electrode base (Metrohm), allow to dry, polish with dry emery paper (grade 2/0, Oakey), remove surface layer with an acetone-soaked tissue, pack the paste into the cavity, allow to dry overnight, polish with dry emery paper (grade 2/0), 3 μm imperial micro finishing film sheet (3M), 0.3 μm imperial micro finishing film sheet (3M), and 0.05 μm alumina particles on a Buehler pad, sonicate for 2 min in water (Anal. Chim. Acta 1995, 300, 5).)

CHROMATOGRAM
Retention time: 11.25
Limit of detection: 0.9 μM

OTHER SUBSTANCES
Also analyzed: arabinose, cellobiose, dextrose, fructose, fucose, galactitol, galactose, galacturonic acid, lactose, lactulose, lyxose, maltose, mannitol, mannose, myo-inositol, raffinose, rhamnose, ribose, sorbose, sucrose

KEY WORDS
orange juice; soft drinks; post-column reaction; fruit

REFERENCE
Huang,X.; Pot,J.J.; Kok,W.T. Determination of sugars by liquid chromatography and amperometric detection with a cuprous oxide modified electrode, *Chromatographia*, **1995**, *40*, 684–689.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 500 μL MeOH, shake for 1 min, centrifuge at 10000 rpm for 1 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 4 Aminex A-27
Mobile phase: 500 mM Boric acid adjusted to pH 8.7 with KOH
Flow rate: 2
Injection volume: 10
Detector: F ex 357 (low-pressure mercury lamp) em 436 (420 nm cutoff filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.8 mm ID stainless steel coil at 150° to the detector. (Reagent was 20 g boric acid and 20 g ethanolamine in 1 L water.)

CHROMATOGRAM
Retention time: 20
Limit of quantitation: 5 nmoles

OTHER SUBSTANCES
Extracted: dextrose, fructose, galactose, maltose, ribose

KEY WORDS
post-column reaction; serum

REFERENCE
Kato,T.; Kinoshita,T. Fluorometric detection and determination of carbohydrates by high-performance liquid chromatography using ethanolamine, *Anal.Biochem.*, **1980**, *106*, 238–243.

SAMPLE
Matrix: bulk
Sample preparation: Evaporate hydrolysates of glycosaminoglycans to dryness, reconstitute in 500 μL 10% benzoic anhydride in pyridine containing 5% 4-dimethylaminopyridine, heat at 37° for 1.5 h, add 4.5 mL water, shake vigorously, pass through a Sep-Pak C18 SPE cartridge three times, wash with 10 mL pyridine:water 10:90, wash with 5 mL water, reverse the direction of flow and elute with 2.5 mL MeCN, evaporate the eluate to dryness, reconstitute with MeCN, centrifuge at 11000 g for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 RP-18
Column: 250 × 4.6 Supelcosil LC-18
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 9.4

OTHER SUBSTANCES
Simultaneous: N-acetylgalactosamine, N-acetylglucosamine, 1,6-anhydroidose, dextrose, fucose, galactosamine, galactose, glucosamine, mannose, 1-methylfucose, 1-methylgalactose, 1-methylmannose, 1-methylxylose
Interfering: 1-methylglucose

KEY WORDS
derivatization; SPE

REFERENCE
Karamanos,N.K.; Hjerpe,A.; Tsegenidis,T.; Engfeldt,B.; Antonopoulos,C.A. Determination of iduronic acid and glucuronic acid in glycosaminoglycans after stoichiometric reduction and depolymerization using high-performance liquid chromatography and ultraviolet detection, *Anal.Biochem.*, **1988**, *172*, 410–419.

SAMPLE
Matrix: carbohydrates
Sample preparation: Mix 10 nmoles total monosaccharides with 200 μL 2 M trifluoroacetic acid, flush with nitrogen for a few min, seal, heat at 100° for 6 h, evaporate to dryness under reduced pressure in a desiccator over NaOH pellets, reconstitute with water, inject an aliquot.

HPLC VARIABLES
Column: 80 × 8 11 μm Hitachi No. 2633 resin (quaternary ammonium)
Mobile phase: Gradient. A was 250 mM pH 8.2 borate buffer. B was 400 mM pH 7.4 borate buffer. C was 600 mM pH 9.3 borate buffer. A:B:C from 100:0:0 to 0:100:0 over 15 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 10 min, maintain at 0:0:100.
Column temperature: 65
Flow rate: 1
Injection volume: 20
Detector: F ex 331 em 383 following post-column reaction. The column effluent mixed with 10% 2-cyanoacetamide in water pumped at 0.25 mL/min and 600 mM pH 9.3 borate buffer pumped at 0.25 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100 ± 0.5° to the detector.

CHROMATOGRAM
Retention time: 58
Limit of detection: 0.1-1 nmole

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fucose, galactose, lyxose, mannose, rhamnose, ribose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Takahashi,M.; Kakehi,K.; Ganno,S. Rapid, automated analysis of monosaccharides by high-performance anion-exchange chromatography of borate complexes with fluorimetric detection using 2-cyanoacetamide, *Anal.Biochem.*, **1981**, *113*, 130–138.

SAMPLE
Matrix: glycoconjugates

Sample preparation: Mix 0.1-1.5 mg glycoconjugate with 200 μL 2 M trifluoroacetic acid, flush with nitrogen for a few min, seal, heat at 100° for 6 h, evaporate to dryness under reduced pressure in a desiccator over NaOH pellets, reconstitute with 200 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Hitachi No. 2633 resin
Mobile phase: Gradient. A was 250 mM pH 8.2 borate buffer. B was 400 mM pH 7.4 borate buffer. C was 600 mM pH 9.3 borate buffer. A:B:C from 100:0:0 to 0:100:0 over 15 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 11 min, maintain at 0:0:100.
Column temperature: 65 ± 1
Flow rate: 1
Injection volume: 20
Detector: UV 276 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide pumped at 0.5 mL/min and 600 mM pH 10.5 borate buffer pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100 ± 0.2° and a 1 m × 0.5 mm ID PTFE cooling coil to the detector.

CHROMATOGRAM
Retention time: 57
Limit of detection: 1 nmole

OTHER SUBSTANCES
Extracted: arabinose, dextrose, fucose, galactose, lyxose, mannose, rhamnose, ribose

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KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Takahashi,M.; Nishimura,Y.; Kakehi,K.; Ganno,S. Sensitive ultraviolet monitoring of aldoses in automated borate complex anion-exchange chromatography with 2-cyanoacetamide, *Anal.Biochem.*, **1981**, *118*, 162–167.

SAMPLE
Matrix: glycoproteins
Sample preparation: 200 μg Glycoprotein + 100 μL water + 100 μL 4 M trifluoroacetic acid, heat at 100° for 6 h, cool to room temperature, evaporate to dryness under reduced pressure at 35°, add 40 μL reagent, heat at 80° for 1 h, cool to room temperature, add 200 μL water, add 200 μL chloroform, vortex vigorously, centrifuge for 1 min, inject an aliquot of the upper aqueous layer. (Prepare the reagent by mixing 165 mg ethyl p-aminobenzoate, 35 mg sodium cyanoborohydride, 41 μL glacial acetic acid, and 350 μL glacial acetic acid.)

HPLC VARIABLES
Column: 150 × 3.9 Pico.Tag (Waters)
Mobile phase: MeCN:MeOH:50 mM pH 4.5 sodium acetate 10:5:85
Column temperature: 45
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: N-acetylgalactosamine, N-acetylglucosamine, 2-deoxyglucose, fucose, galactosamine, galactose, glucosamine, glucose, lactose, maltose, mannose

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KEY WORDS
derivatization

REFERENCE
Kwon,H.; Kim,J. Determination of monosaccharides in glycoproteins by reverse-phase high-performance liquid chromatography, *Anal.Biochem.*, **1993**, *215*, 243–252.

SAMPLE
Matrix: plants
Sample preparation: Condition trimethylaminopropylsilica SAX and cyclohexylsilica SPE cartridges (Analytichem) with 4 mL MeOH and 4 mL water. Heat 1 g plant material with 10 mL EtOH:water 80:20 in a sealed tube at 100° for 15-30 min, evaporate the extract to dryness, reconstitute with water, pass through the SPE cartridges, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 6.5 Sugar Pak-1 microparticulate gel, calcium form (Waters)
Mobile phase: 100 μM Calcium EDTA
Column temperature: 70
Flow rate: 0.4
Injection volume: 50
Detector: F ex 360 em 470 following post-column reaction. The column effluent mixed with 30 mM benzamidine in 1 M KOH pumped at 1 mL/min and the mixture flowed through a 530 μL reaction coil (Varian PCR1) at 100° to the detector.

CHROMATOGRAM
Retention time: 10.92
Limit of detection: 15.8-62.5

OTHER SUBSTANCES
Extracted: arabinose, dextrose, fructose, fucose, galactose, lactose, mannose
Interfering: rhamnose

KEY WORDS
post-column reaction; SPE

REFERENCE
Coquet,A.; Veuthey,J.-L.; Haerdi,W. Selective post-column fluorigenic reaction with benzamidine for trace level detection of reducing saccharides in liquid chromatography, *J.Chromatogr.*, **1991**, *553*, 255–263.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 160 × 4 8 μm DAX8 anion-exchange resin, sulfate form (Durrum Chemical Co.) (Regenerate resin outside the column by washing 10 g resin with 400 mL water, 400 mL 500 mM NaCl at 50°, water, 0.5 N sodium sulfate at 50° (until a negative chloride test is obtained), water, and EtOH:water 95:5. Slurry pack below 70° with mobile phase at 1.14 mL/min.)
Mobile phase: EtOH:water 87.6:12.4
Column temperature: 88
Flow rate: 0.57
Injection volume: 5-100
Detector: UV 562 following post-column reaction. The column effluent mixed with the reagent pumped at 0.3 mL/min and the mixture flowed for 5 min through a coil of 0.3 mm ID PTFE at 100° to the detector. (Prepare reagent by mixing equal volumes of solution A and solution B, the mixture is stable for at least 1 month. Solution A is 1 g of copper sulfate pentahydrate and 3.7 g aspartic acid in 1 L water. Solution B is 38 g sodium carbonate decahydrate and 2 g sodium bicinchoninate (Pierce Chemical Co.) in 1 L water.)

CHROMATOGRAM
Retention time: 83
Limit of detection: <500 pmole

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, digitose, fructose, fucose, galactose, 2-d-galactose, 6-d-glucose, gulose, lyxose, mannose, 3-O-methylglucose, rhamnose, 2-d-ribose, ribose, sorbose, tagatose

KEY WORDS
post-column reaction

REFERENCE
Mopper,K. Improved chromatographic separations on anion-exchange resins. I. Partition chromatography of sugars in ethanol, *Anal.Biochem.*, **1978**, *85*, 528–532.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: 150 × 6 6 μm Shodex RSPak DC-613 sulfonated polystyrene 55% cross-linked with divinylbenzene calcium form (Showa Denko)
Mobile phase: MeCN:water 80:20
Column temperature: 4
Flow rate: 0.5
Injection volume: 20
Detector: UV 280 following post-column reaction. The column effluent mixed with 500 mM pH 8.5 borate buffer pumped at 0.5 mL/min and 1% 2-cyanoacetamide in water pumped at 0.5 mL/min and the mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 100 ± 1° and a 1 m × 0.5 mm PTFE cooling coil to the detector.

CHROMATOGRAM
Retention time: k' 2.68 (α-D-xylose), k' 2.20 (β-D-xylose)

OTHER SUBSTANCES
Also analyzed: allose, altrose, arabinose, dextrose, fucose, galactose, gulose, idose, lyxose, mannose, rhamnose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Suzuki,S.; Kakehi,K. Improved analysis of aldose anomers by high-performance liquid chromatography on cation-exchange columns, *J.Chromatogr.*, **1984**, *291*, 317–325.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 6 μm Shodex RSPak DC-613 sulfonated polystyrene 55% cross-linked with divinylbenzene (H^+) (Showa Denko)
Mobile phase: MeCN:water 90:10
Flow rate: 0.6
Injection volume: 20
Detector: E, Irika E-502, glassy carbon working electrode 0.30 V, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 200 mM pH 9.5 borate buffer pumped at 0.25 mL/min and 1.5% 2-cyanoacetamide in water pumped at 0.25 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100° and a 1 m × 0.5 mm PTFE cooling coil to the detector.

CHROMATOGRAM
Retention time: 13
Limit of detection: 20 pmole

OTHER SUBSTANCES
Simultaneous: fucose, galactose, rhamnose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Konishi,T.; Suzuki,S. Electrochemical detection of reducing carbohydrates in high-performance liquid chromatography after post-column derivatization with 2-cyanoacetamide, *J.Chromatogr.*, **1984**, *299*, 245–251.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 3 mL MeCN and 5 mL water. Mix 250 μL of an aqueous solution with 225 μL 1% dansylhydrazine in EtOH and 45 μL 10%trichloroacetic acid in water, heat at 65° for 20 min, dilute with water to an organic solvent concentration of ≤5%, add a 5 mL aliquot to the SPE cartridge, wash with 5 mL MeCN:water 5:95 at ≤2 mL/min, elute with 6 mL MeCN:water 20:80 at ≤2 mL/min (J. Chromatogr. 1983, 256, 27), lyophilize the eluate, reconstitute with MeCN:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm 600 RPB C18 (Alltech)
Mobile phase: MeCN:water 20:80 containing 10 mM formic acid, 40 mM acetic acid, and 1 mM triethylamine. (After each run flush column with MeCN:MeOH 20:80 for 5 min.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Limit of detection: 200-300 pmole

OTHER SUBSTANCES
Simultaneous: dextrose, fucose, galactose, lyxose, mannose

KEY WORDS
derivatization; SPE

REFERENCE
Eggert,F.M.; Jones,M. Measurement of neutral sugars in glycoproteins as dansyl derivatives by automated high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *333*, 123–131.

SAMPLE
Matrix: solutions
Sample preparation: Heat 100-200 pmole sample with 20 μL 4 M trifluoroacetic acid and 20 μL 4 M HCl in a tube sealed under vacuum at 100° for 6 h, add 500 pmole L-rhamnose, evaporate to dryness under reduced pressure at 50°, add 50 μL 9.8% sodium bicarbonate solution (freshly prepared), add 2 μL acetic anhydride, let stand at room temperature with occasional stirring for 30 min, add 200 μL 100-200 mesh Dowex 50W-X2 (H^+), check that pH is about 3. Add the mixture to a 100 × 5 column and wash it with 5 bed volumes of water, evaporate to dryness under reduced pressure, add 5 μL reagent, seal tube, heat at 100° for 13-15 min, add 2 μL 20 mg/mL sodium cyanoborohydride in water (freshly prepared), reseal the tube, heat at 90° for 8 h, dilute with 20 μL water, inject the whole amount on to a 600 × 7.5 10 μm TSK-GEL G2000PW column (Toyo Soda) and elute with 20 mM pH 7.5 ammonium acetate buffer at 0.5 mL/min, collect the sugar fraction at 40-55 min. Evaporate the eluate to dryness and reconstitute it with 250 μL water, inject a 5 μL aliquot. (Prepare reagent by mixing 500 mg 2-aminopyridine, 400 μL concentrated HCl, and 11 mL water.)

HPLC VARIABLES
Column: two 250 × 4.6 5 μm Ultrasphere-ODS column in series
Mobile phase: MeCN:250 mM pH 4.0 sodium citrate buffer 1:99
Flow rate: 0.5
Injection volume: 5
Detector: F ex 320 em 400

CHROMATOGRAM
Retention time: 39

Internal standard: L-rhamnose
Limit of quantitation: 10 pmoles

OTHER SUBSTANCES
Simultaneous: N-acetyl-D-mannosamine, N-acetylgalactosamine, N-acetylglucosamine, 2-deoxy-D-ribose, dextrose, fucose, galactose, mannnose, ribose

KEY WORDS
derivatization; SPE

REFERENCE
Takemoto,H.; Hase,S.; Ikenaka,T. Microquantitative analysis of neutral and amino sugars as fluorescent pyridylamino derivatives by high-performance liquid chromatography, *Anal.Biochem.*, **1985**, *145*, 245–250.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 80 × 3 Hitachi 2633
Mobile phase: 700 mM pH 8.5 Borate buffer containing 0.01% EDTA
Column temperature: 60
Flow rate: 0.7
Injection volume: 20
Detector: E, Irika E-502, glassy carbon working electrode 350 mV, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 100 mM ethylenediamine sulfate pumped at 0.25 mL/min and 700 mM pH 9.0 borate buffer pumped at 0.25 mL/min and the mixture flowed through a 30 m × 0.5 mm ID PTFE coil at 140° and a 10 m × 0.2 mm ID cooling coil to the detector.

CHROMATOGRAM
Retention time: 30
Limit of detection: 1 pmole

OTHER SUBSTANCES
Simultaneous: dextrose, galactose, mannose, rhamnose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Enami,K.; Konishi,T.; Suzuki,S.; Kakehi,K. Use of ethylenediamine sulphate for post-column derivatization of reducing carbohydrates to electrochemically oxidizable compounds in high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *361*, 321–329.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: HPIC-AS6 anion-exchange (Dionex)
Mobile phase: 150 mM NaOH
Column temperature: 36
Flow rate: 0.5
Injection volume: 20
Detector: UV 500 following post-column reaction. The column effluent mixed with the reagent pumped at 0.2 mL/min (?) and the mixture flowed through a knitted 10 m × 0.3 mm ID PTFE coil at 90° and then a short knitted PTFE coil at 22° to the detector. (Reagent was 2 mg/mL thymol in concentrated sulfuric acid, let stand for 30 min after preparation, discard after 48 h. (The reagent was displaced from a pressure vessel into the post-column reaction system by pumping n-heptane into the vessel.)

CHROMATOGRAM
Retention time: 9.3
Limit of detection: 100 ng

OTHER SUBSTANCES
Simultaneous: arabinose, desoxyribose, dextrose, fructose, galactose, lactose, maltose, mannose, raffinose, ribose, saccharose
Noninterfering: methyl arabinose, methyl glycose, rhamnose, rutinose, trehalose

KEY WORDS
post-column reaction

REFERENCE
Engelhardt,H.; Ohs,P. Trace analysis of sugars by HPLC and post-column derivatization, *Chromatographia*, **1987**, *23*, 657–662.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve 50 mg sugars in 700 μL pyridine, add 700 μL 720 mM hydroxylamine hydrochloride in pyridine, heat at 60° for 10 min, add 250 μL acetic anhydride, heat at 75° for 10 min, evaporate to dryness under reduced pressure, reconstitute with 3 mL chloroform. Wash the organic layer three times with 6 mL portions of water and dry it over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, take up in chloroform, pass through silica gel using chloroform, evaporate the eluate to dryness, reconstitute, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm μBondapak C18
Mobile phase: Gradient. MeCN:water from 35:75 to 50:50 over 15 min.
Flow rate: 1
Injection volume: 5
Detector: UV 207

CHROMATOGRAM
Retention time: k' 3.1
Limit of detection: 3 μg

OTHER SUBSTANCES
Also analyzed: allose, altrose, arabinose, dextrose, fructose, galactose, gulose, idose, lyxose, mannose, ribose, talose

KEY WORDS
derivatization

REFERENCE
Velasco,D.; Castells,J.; Lopez-Calahorra,F. High-performance liquid chromatographic separation of monosaccharides as their peracetylated ketoximes and aldononitriles, *J.Chromatogr.*, **1990**, *519*, 228–236.

SAMPLE
Matrix: solutions
Sample preparation: 10 μL EtOH containing sugars + 110 μL EtOH:acetic acid 99.9:0.1 + 100 μL Fmoc-hydrazine in MeCN, mix, heat at 65° for 3 h, cool to room temperature, dilute with EtOH or EtOH:acetic acid 99.9:0.1, inject an aliquot. (Prepare Fmoc-hydrazine as follows. Dissolve 100 mg 9-fluorenylmethylchloroformate in 25 mL MeCN, add this solution dropwise with stirring to 1 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!), stir for 30 min, evaporate under reduced pressure, use the crude product or recrystallize from EtOH or MeCN (mp 173-5°). Prepare a solution of Fmoc-hydrazine in MeCN so that the hydrazine:sugar ratio is 10:1.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax ODS

Mobile phase: Gradient. A was MeCN:water 27:73 containing 80 mM acetic acid. B was MeCN:water 30:70 containing 80 mM acetic acid. A:B from 100:0 to 0:100 over 30 min.
Flow rate: 1
Detector: F ex 270 em 320

CHROMATOGRAM
Retention time: 15
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Simultaneous: fructose, galactose, lactose, maltose, mannose, ribose

KEY WORDS
derivatization

REFERENCE
Zhang,R.-E.; Cao,Y.-L.; Hearn,M.W. Synthesis and application of Fmoc-hydrazine for the quantitative determination of saccharides by reversed-phase high-performance liquid chromatography in the low and subpicomole range, *Anal.Biochem.*, **1991**, *195*, 160–167.

SAMPLE
Matrix: solutions
Sample preparation: Add 55 μL phenylisocyanate to a 1 mg/mL solution in DMF, heat at 55° for 95 min, cool, add 500 μL MeOH, let stand for 5 min, make up to 6 mL with DMF, dilute an aliquot 10-fold with DMF, inject an aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm ODS 224 RP18 (Brownlee)
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 9.42
Limit of detection: 0.2 ng

OTHER SUBSTANCES
Simultaneous: allose, arabinose, deoxyglucose, deoxyribose, dextrose, fucose, galactose, lyxose, mannose, methylgalactoside, methylglucoside, methylmannoside, rhamnose, ribose

KEY WORDS
derivatization; more than one derivative was observed, retention time is for major derivative

REFERENCE
Rakotomanga,S.; Baillet,A.; Pellerin,F.; Baylocq-Ferrier,D. Liquid chromatographic analysis of monosaccharides with phenylisocyanate derivatization, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 587–591.

SAMPLE
Matrix: solutions
Sample preparation: Add 25 nmoles 3-O-methylglucose, evaporate the solution to dryness, add 50 μL 300 mM sodium cyanoborohydride in 2 M pH 7.0 ammonium acetate (freshly prepared), heat at 105° for 4 h, add 100 μL water, add 40 μL 6 M formic acid, evaporate to dryness under reduced pressure, add 500 μL MeOH, evaporate to dryness, repeat MeOH evaporation twice more, add 100 μL EtOH:water:triethylamine 40:40:20, evaporate to dryness, add 100 μL EtOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under reduced pressure, reconstitute with 20 μL MeCN:water 60:40, add 180 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C18

Mobile phase: Gradient. A was 50 mM pH 6.8 ammonium acetate. B was 100 mM pH 6.8 ammonium acetate in MeCN:MeOH:water 44:10:46. A:B 78:22 until the run is over, to 0:100 over 5 min, maintain at 0:100 for 6 min, return to initial conditions over 5 min
Column temperature: 30
Flow rate: 0.8
Detector: UV 254

CHROMATOGRAM
Retention time: 13.5
Internal standard: 3-O-methylglucose (20)
Limit of detection: 50 pmole

OTHER SUBSTANCES
Simultaneous: dextrose, fucose, galactose, mannose, ribose

KEY WORDS
derivatization

REFERENCE
Spiro,M.J.; Spiro,R.G. Monosaccharide determination of glycoconjugates by reverse-phase high-performance liquid chromatography of their phenylthiocarbamyl derivatives, *Anal.Biochem.*, **1992**, *204*, 152–157.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 7.8 Aminex HPX-87P cation-exchange (Bio-Rad)
Mobile phase: Water
Column temperature: 85
Flow rate: 0.8
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 20 m × 0.3 mm ID PTFE coil at 90° and a coil at 0° to the detector. (Reagent was prepared from 4000 ppm Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) in 2 M NaOH (A) and 40 mM hydrogen peroxide (B). A:B 70:30.)

CHROMATOGRAM
Retention time: 16.5
Limit of detection: 30 ng

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fructose, galactose, mannose, ribose

KEY WORDS
post-column reaction

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Hernandez,V.; Toribio,L.; Mendez,R. Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) as a reagent for post-column derivatization of neutral monosaccharides in high pressure liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 1105–1116.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a 500 μM saccharide solution in MeCN:water 30:70 with 50 μL 10 mM reagent in MeCN and 100 μL 0.5% trichloroacetic acid in MeCN, heat at 65° in the dark for 3 h. Remove a 50 μL aliquot of the reaction mixture, add 200 μL water, add 200 μL ethyl acetate, mix, centrifuge at 3000 rpm for 2 min, repeat the ethyl acetate wash twice more. Dry the aqueous layer under reduced pressure, reconstitute with 200 μL MeCN, inject an aliquot. (Synthesis of reagent, R-(+)-DBD-ProCZ, is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the

flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro- 2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Add 100 mg DBD-F in 10 mL MeCN to 47 mg R-(+)-proline in 20 mL 250 mM pH 11.5 sodium carbonate solution, stir at room temperature for 30 min, wash with ethyl acetate, adjust the pH of the aqueous layer to 1-2 with 2 M HCl, extract three times with 30 mL ethyl acetate. Combine the extracts and evaporate them under reduced pressure, recrystallize from benzene/ethyl acetate to give R-(+)-4-(N,N-dimethylaminosulfonyl)-7-(2-carboxypyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-Pro) as yellow needles (mp 187-9° d) (Analyst 1989, 114, 1233). Suspend 55 mg (R)-(+)-DBD-Pro in 55 mL anhydrous diethyl ether at 0°, add 110 mg phosphorus pentachloride, stir at 5° for 1 h, filter quickly, evaporate to dryness under reduced pressure, dry under vacuum over phosphorus pentoxide for 12 h to give R-(+)-4-(N,N-dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-Pro-Cl) as yellow crystals (mp 116-17°) (Analyst 1993, 118, 759). Add 130 mg DBD-Pro-Cl dissolved in 25 mL anhydrous benzene dropwise to 100 mL MeOH containing 70 mg hydrazine hydrate, stir for 30 min at room temperature, evaporate under reduced pressure, recrystallize from ethyl acetate:MeOH 90:10 to give R-(+)-4-(2-carbazolylpyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (R-(+)-DBD-ProCZ) as orange crystals (mp 107-109°) (Anal. Proc. 1994, 31, 265).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water 15:85
Column temperature: 40
Flow rate: 1
Detector: F ex 450 em 540

CHROMATOGRAM
Retention time: 15.81

OTHER SUBSTANCES
Simultaneous: N-acetyl-D-glucosamine, arabinose, dextrose, galactose, mannose

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KEY WORDS
derivatization

REFERENCE

Toyo'oka,T.; Kuze,A. Determination of saccharides labelled with a fluorescent reagent, DBD-ProCZ, by liquid chromatography, *Biomed.Chromatogr.*, **1997**, *11*, 132–136.

SAMPLE

Matrix: urine

Sample preparation: Mix acetone with urine so as to make a 63:47 acetone:urine mixture, centrifuge a 6 mL aliquot. Evaporate the acetone from the supernatant under a stream of helium at 35°, add 30 mg Dowex 50W-X8, add 30 mg Dowex 1-X8, agitate, centrifuge, inject a 1-10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.3 Hitachi 3013 N anion-exchange resin, phosphate form
Mobile phase: MeCN:water 83:17
Column temperature: 60
Flow rate: 1
Injection volume: 1-10
Detector: UV 530 following post-column reaction. The column effluent mixed with the reagent pumped at 1.5 mL/min, the mixture flowed through a 3 m × 0.5 mm i.d. coil of PTFE tubing at 85° and a 1 m × 0.5 mm i.d. coil of PTFE tubing at room temperature to the detector. (Reagent was 2 g/L blue tetrazolium in EtOH:water 50:50 containing 180 mM NaOH.)

CHROMATOGRAM

Retention time: 10
Limit of detection: 10 ng

OTHER SUBSTANCES

Extracted: arabinose, dextrose, fructose, fucose, galactose, lactose, ribose

KEY WORDS

post-column reaction

REFERENCE

D'Amboise,M.; Hanai,T.; Noël,D. Liquid-chromatographic measurement of urinary monosaccharides, *Clin. Chem.*, **1980**, *26*, 1348–1350.

SAMPLE

Matrix: urine

Sample preparation: 100 μL Urine + 10 μL 10% trichloroacetic acid in water + 50 μL 5% dansyl hydrazine in MeCN, heat at 65° for 20 min, cool in ice, add an equal volume of water, inject a 10-300 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeCN:80 mM acetic acid 21:79
Flow rate: 1
Injection volume: 10
Detector: F ex 360 em >470

CHROMATOGRAM

Retention time: 11.5
Limit of detection: 5-15 pmole

OTHER SUBSTANCES

Extracted: cellobiose, 2-deoxyglucose, 2-deoxyribose, dextrose, fucose, galactose, gentobiose, lactose, maltose, mannose, rhamnose, ribose
Interfering: arabinose, fructose

KEY WORDS

derivatization

REFERENCE
Mopper,K.; Johnson,L. Reversed-phase liquid chromatographic analysis of Dns-sugars. Optimization of derivatization and chromatographic procedures and applications to natural samples, *J.Chromatogr.*, **1983**, *256*, 27–38.

SAMPLE
Matrix: urine
Sample preparation: 10 μL Urine + 200 μL reagent, heat at 65° for 16 h, cool to room temperature, inject a 5 μL aliquot of the clear supernatant. (Prepare reagent by dissolving 5 mg Fmoc-hydrazine in 1 mL MeCN, add 10 μL buffer. Buffer was 1.44 M formic acid containing 600 mM NaOH. Prepare Fmoc-hydrazine as follows. Dissolve 1 g 9-fluorenylmethyl chloroformate in 100 mL EtOH, add this solution dropwise with stirring to 10 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!), stir for 30 min, filter off the precipitate, wash it twice with 20 mL portions of ice-cold EtOH, dry at room temperature.)

HPLC VARIABLES
Guard column: 10 × 4.6 3 μm Spherisorb ODS II
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. Isopropanol:isobutyl alcohol:water 6:6:88 for 13 min, to 80:0:20 (step gradient), maintain at 80:0:20 for 6 min, re-equilibrate at initial conditions.
Column temperature: 50
Injection volume: 5
Detector: F ex 270 em 315

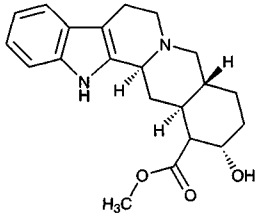
CHROMATOGRAM
Retention time: 9.8
Limit of detection: 20 nM

OTHER SUBSTANCES
Extracted: lactulose, 3-O-methyl-D-glucose, rhamnose

KEY WORDS
derivatization

REFERENCE
Rooyakkers,D.R.; van Eijk,H.M.H.; Deutz,N.E.P. Simple and sensitive multi-sugar-probe gut permeability test by high-performance liquid chromatography with fluorescence labelling, *J.Chromatogr.A*, **1996**, *730*, 99–105.

Yohimbine



Molecular formula: $C_{21}H_{26}N_2O_3$
Molecular weight: 354.45
CAS Registry No.: 146-48-5
Merck Index: 10236

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18